(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 June 2002 (06.06.2002)

PCT

(10) International Publication Number
WO 02/43486 A1

(51) International Patent Classification[7]: A01N 1/00

(21) International Application Number: PCT/US01/45237

(22) International Filing Date:
29 November 2001 (29.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/253,785	29 November 2000 (29.11.2000)	US
60/253,787	29 November 2000 (29.11.2000)	US

(71) Applicants *(for all designated States except US)*: **XY, INC.** [US/US]; 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). **COLORADO STATE UNIVERSITY** [US/US]; Colorado State University Research Foundation, Room 410, 601 South Howes Street, Fort Collins, CO 80522 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SEIDEL, George, E.** [US/US]; c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). **SUH, Tae, Kwang** [US/US]; c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). **LU, Kehuan** [US/US]; c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US).

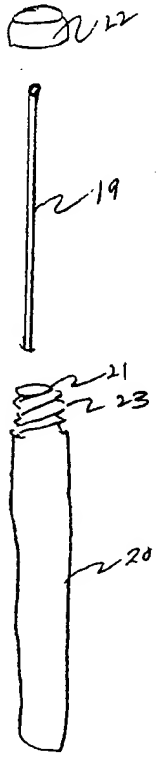
(74) Agent: **MILES, Craig, R.**; Santangelo Law Offices, P.C., Third Floor, 125 South Howes, Fort Collins, CO 80521 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE,

[Continued on next page]

(54) Title: SYSTEM FOR IN-VITRO FERTILIZATION WITH SPERMATOZOA SEPARATED INTO X-CHROMOSOME AND Y-CHROMOSOME BEARING POPULATIONS

(57) Abstract: An IVF system for successfully utilizing spermatozoa separated into X-chromosome bearing and into Y-chromosome bearing population for insemination. The IVF system includes fertilization medium that can shorten the time from insemination to cleavage and a portable incubator for the transportation of maturing oocytes and inseminated oocytes comprising a straw (19) and an incubation element (20) that can be sealed with a cap (22).



WO 02/43486 A1

GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SYSTEM FOR IN-VITRO FERTILIZATION WITH SPERMATOZOA SEPARATED INTO X-CHROMOSOME AND Y-CHROMOSOME BEARING POPULATIONS

This application claims the benefit of United States Provisional Patent Application No. 60/253,787, filed November 29, 2000 and United States Provisional Patent Application No. 60/253,785, filed November 29, 2000, each hereby incorporated by reference herein.

I. TECHNICAL FEILD

Devices, compositions, and methods that improve the quality of embryos generated using in-vitro fertilization (IVF) with spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations.

II. BACKGROUND

An attractive feature of IVF is that many fewer spermatozoa can be required for insemination than for artificial insemination. However, IVF using spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations (separated spermatozoa) can necessitate modifications to conventional IVF techniques. This may due in part to the pre-capacitation of such spermatozoa.

In most cases, the percentages of oocytes (oocyte, ootid, or ova, or plurality of same as appropriate to the application) fertilized with separated and unseparated spermatozoa are similar, and events during the first cell cycle are timed similarly for separated and unseparated spermatozoa. However, with conventional procedures, blastocyst production with separated spermatozoa can be 70%-90% of controls with spermatozoa that have not been separated. For example, development to blastocysts has been shown to be 17% with bovine oocytes inseminated with separated spermatozoa, compared with >25% which might be expected with IVF using unseparated spermatozoa as described in the journal article entitled "In Vitro Fertilization With Flow-Cytometerically-Sorted Bovine Sperm" Theriogenology 52: 1393-1405 (1999), hereby incorporated by reference.

Several factors may contribute to these results. One factor may be that staining of sperm with Hoechst 33342 appears to cause a decline in motility of spermatozoa. Another factor, may be the physical forces the spermatozoa are subject to during the separation process. As but one example, in flow cytometric separation of spermatozoa, spermatozoa exit the flow cytometer at nearly 100 km/h before impacting on the surface of the collection medium. During transit through the flow cytometer spermatozoa can be subjected to laser light at an intensity of over 100mW. While the transit time may only be 1-2μsec, this may affect the spermatozoal DNA, and thus, also effect subsequent embryonic development. The process of separating sperm with flow cytometry can also result in a highly diluted sample, 600,000 spermatozoa/mL or less, and subsequent centrifugation steps are necessary to provide concentrated spermatozoa suitable for insemination.

Another problem with utilizing separated spermatozoa in IVF techniques may be that the facility in which the spermatozoa are separated may be in a different location than where the male mammal from which the spermatozoa are collected is located, which may be different from where the female mammal from which the oocytes are collected is located, which may be a different location from where the in-vitro fertilization is to occur, and which may be a different location from where the female mammal into which the in-vitro cultured embryos are to be transferred. Conventionally, separated sperm may be cryopreserved and transported frozen to the facility at which the IVF techniques are administered. Maturing oocytes are conventionally transported to the facility at which the IVF techniques are administered in portable incubation systems. The maturing oocytes are then inseminated with previously frozen-thawed sperm cells. To avoid cryopreservation of sperm cells or as a convenience to the various facilities involved it may be beneficial to transport maturing oocytes directly to the facility separating the spermatozoa so that separated sperm cells can be added to the oocytes without cryopreservation. However, conventional IVF and in vitro culture of the resulting zygotes typically comprises a separate set of apparatus and procedures making it inconvenient, difficult, or impossible to inseminate and culture oocytes in the same facility in which spermatozoa are separated.

Even though X-chromosome bearing spermatozoa and Y-chromosome bearing spermatozoa have been differentiated by and separated based upon the difference in emitted fluorescence for many years, and even though separated spermatozoa have been used for some time with IVF techniques, and even though there is large commercial market for embryos produced with IVF techniques and separated spermatozoa, the above-mentioned problems have yet to be resolved.

As to the problems with conventional techniques of IVF using separated spermatozoa, and specifically separated spermatozoa, stained spermatozoa, or spermatozoa that are from previously frozen sperm, and with conventional strategies involving the transportation of separated sperm and maturing oocytes, the invention addresses each in a practical manner.

III. DISCLOSURE OF THE INVENTION

Accordingly, one of the broad objects of particular embodiments of the invention can be to provide devices, compositions and methods that provide transportation of inseminated oocytes, promotes cleavage of fertilized oocytes and improves the quality of embryos generated with techniques utilizing spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations.

Another broad object of particular embodiments of the invention can be to provide devices, compositions, and methods that promote cleavage and improve quality of embryos generated using IVF with spermatozoa that are derived from previously frozen sperm.

Another broad object of particular embodiments of the invention can be to provide devices, compositions, and methods that promote cleavage and improve quality of embryos generated using IVF with spermatozoa that have previously been stained with a DNA binding fluorochrome.

Another broad object of particular embodiments of the invention can be to provide medium for embryonic culturing that can contain non-essential amino acids.

Another broad object of the invention can be to provide apparatus and methods for transporting maturing oocytes and fertilized oocytes for the convenience of the end user(s) or to avoid cryopreservation of the spermatozoa used to fertilize oocytes.

Naturally further objects of the invention are disclosed throughout other areas of specification.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an embodiment of the invention in which spermatozoa from fresh or previously frozen-thawed sperm are stained.

Figure 2 shows an embodiment of the invention for separating stained spermatozoa in to X-chromosome bearing and Y-chromosome bearing populations.

Figure 3 shows another view of an embodiment of the invention for separating stained spermatozoa in to X-chromosome bearing and Y-chromosome bearing populations.

Figure 4 shows an embodiment of a portable incubation system in which oocytes can be fertilized.

V. MODE(S) FOR CARRYING OUT THE INVENTION

The invention involves devices, methods, and compositions for the in-vitro insemination and fertilization of oocytes (oocyte, ootid, or ova, or plurality of same as appropriate to the application) and the culture of embryos resulting from such techniques.

Embodiments of the invention can include fresh spermatozoa, or spermatozoa from frozen-thawed sperm of numerous species of mammals. The invention should be understood not to be limited to the species of mammals cited by the specific examples within this patent application. Embodiments of the invention, for example, may include fresh spermatozoa or spermatozoa from frozen-thawed sperm of animals having

commercial value for meat or dairy production such as swine, bovids, ovids, equids, buffalo, or the like (naturally the mammals used for meat or dairy production may vary from culture to culture). It may also include fresh spermatozoa or spermatozoa from frozen-thawed sperm from individuals having rare or uncommon attribute(s), such as morphological characteristics including weight, size, or conformation, or other desired characteristics such as speed, agility, intellect, or the like. It may include frozen-thawed sperm from deceased donors, or fresh or frozen-thawed spermatozoa from rare or exotic mammals, such as zoological specimens or endangered species. Embodiments of the invention may also include fresh or frozen-thawed spermatozoa collected from primates, including but not limited to, humans, chimpanzees, gorillas, or the like, and may also include fresh or frozen-thawed spermatozoa from marine mammals, such as whales or porpoises.

Now referring primarily to Figure 1, in some embodiments of the invention, Hoechst 33342 stain (1) can be added to bovine spermatozoa contained in frozen-thawed sperm (2) to establish a concentration of 224μM. The incubation time of the spermatozoa contained in the frozen-thawed sperm (2) with the stain (1) can be about 190 minutes. In anther embodiment of the invention, the stain (1) can be added to the bovine sperm (2) to establish a concentration of 2240μM and then incubated for about 60 minutes. Frozen-thawed sperm treated in either manner can improve the resolution of X-chromosome bearing from Y-chromosome bearing spermatozoa. Understandably, from application to application (such as frozen-thawed sperm from different species) the amount of incubation time and the specific concentration of stain can adjusted to optimize the resolution of the X-chromosome bearing from Y-chromosome bearing spermatozoa.

With respect to the cleavage rates of inseminated oocyte(s), the increase in stain concentration up to at least 10X does not appear to have a depressive effect on either cleavage or embryonic development. Higher stain concentrations may actually be beneficial with respect to certain applications because the length of incubation time may be decreased improving percent cleavage. From application to application length of incubation time can be adjusted to optimize cleavage results or embryonic development, as desired.

Now referring primarily to Figures 2 and 3, a flow cytometer embodiment of the invention is shown which includes a sperm cell source (3) which acts to establish or supply stained spermatozoa or other type of stained cells to be analyzed by the flow cytometer. The sperm cells are deposited within a nozzle (4) in a manner such that the cells are surrounded by a sheath fluid (5). The sheath fluid (5) is usually supplied by some sheath fluid source (6) so that as the cell source (3) supplies sperm cells, the sheath fluid (5) is concurrently fed through the nozzle (4). In this manner it can be easily understood how the sheath fluid (5) forms a sheath fluid environment for the cells. Since the various fluids are provided to the flow cytometer at some pressure, they flow out of the nozzle (4) and exit at the nozzle orifice (7). By providing some type of oscillator (8) which may be very precisely controlled through an oscillator control (9), pressure waves may be established within the nozzle (4) and transmitted to the fluids exiting the nozzle (4) at nozzle orifice (7). Since the oscillator (9) thus acts upon the sheath fluid (5), the stream (10) exiting the nozzle orifice (7) eventually and regularly forms drops (11). Because the sperm cells are surrounded by a sheath fluid environment, the drops (11) may contain within them individually isolated (generally) cells or other items.

Since the drops (11) generally contain isolated sperm cells, the flow cytometer can distinguish and separate droplets based upon whether or not the appropriate sperm cell is contained within the drop. This is accomplished through a cell sensing system (12). The cell sensing system involves at least some type of sensor (13) which responds to the cells contained within each drop (11) as described by U.S. Patent No. 5135759, hereby incorporated by reference. As the Johnson patent explains for spermatozoa or sperm cells, although the staining and separation inventions can be understood to be used with a variety of frozen-thawed cells, the cell sensing system (13) may cause an action depending upon the relative presence or relative absence of the bound fluorochrome which may be excited by some stimulant such as the laser exciter (14). While each type of sperm cell can be stained by the stain or fluorochrome, as described above, the differing length of the X-chromosome and the Y-chromosome causes different amounts of stain to be bound, Thus, by sensing the degree of fluorescence emitted by the fluorochrome upon excitation it is possible to discriminate between X-bearing spermatozoa and Y-bearing spermatozoa by their differing fluoresence emission levels.

In order to achieve separation and isolation of the appropriate sperm cells, the signals received by sensor (14) are fed to some type of sorter discrimination system (15) which very rapidly makes a differentiation decision and can differentially charge each drop (11) based upon whether it has decided that the desired sperm cell does or does not exist within that drop (11). In this manner the separation or discrimination system (15) acts to permit the electrostatic deflection plates (16) to deflect drops (11) based on whether or not they contain the appropriate sperm cell. As a result, the flow cytometer acts to sort the sperm cells by causing them to land in one or more collectors (17). Thus by sensing some property of the sperm cells the flow cytometer can discriminate between sperm cells based on a particular characteristic and place them in the appropriate collector (17). In the system presently used to sort spermatozoa, the X-bearing spermatozoa droplets are charged positively and thus deflect in one direction, the Y-bearing spermatozoa droplets are charged negatively and thus deflect the other way, and the wasted stream (that is unsortable cells) is uncharged and thus is collected in an undeflected stream into a suction tube or the like.

Now referring primarily to Figure 3, the process can be even further understood. As shown in that figure, the nozzle (4) emits a stream (10) which because of the oscillator (8) (not shown in Figure 3) forms drops (11). Since the cell source (3) (not shown in Figure 3) may supply sperm cells (1) which have been stained according the invention, the magnitude of the fluorescent emission stimulated by the laser exciter (13) is differentially determined by sensor (14) so that the existence or nonexistence of a charge on each drop (11) as it separates from stream (10) can be controlled by the flow cytometer. This control results in positively charged, negatively charged, and uncharged drops based upon the encapsulated sperm cell. As shown in Figure 3, certain drops are shown as deflected drops (18). These deflected drops (18) are those containing sperm cells (2) differentiated by bearing an X-chromosome or a Y-chromosome. Separated sperm are then deposited in the appropriate collector (17) for later use. See also, International Patent Application PCT/US98/27909, hereby incorporated by reference.

While the above description focuses on the separation of spermatozoa with flow cytometry, separation of X-chromosome bearing spermatozoa and Y-chromosome

bearing spermatozoa based upon the difference in measurable fluorescent emission may also include numerous other technologies such as liquid chromatography, gel electrophoresis, and other technologies that similarly excite the amount of bound fluorochrome to differentiate between X chromosome bearing spermatozoa and the Y chromosome bearing spermatozoa.

Embodiments of the invention can also comprise collecting oocytes from a female mammal. With respect to certain embodiments of the invention, oocytes can be aspirated from the ovaries of the desired female mammal or can be obtained from slaughterhouse ovaries. The oocytes can be matured in TCM199 supplemented with about 10% fetal calf serum plus hormones (15 ng FSH, 1 μg LH, 1 μg E_2/ml) for 22-24 h at 39°C, in about 5% CO_2 in air.

Ten to 15 oocytes can be transferred to a 50 μl drop of fertilization medium containing non-essential amino acids, such as tyrode albumin lactaate pyruvate (TALP) supplemented with non-essential amino acids derived from Eagles Medium, and which can further contain 0.6% bovine serum albumin, 20 μg heparin/ mL and 5 mM caffeine. Alternately, oocytes can be fertilized in other medium containing non-essential amino acids such as the chemically defined medium described in the journal article entitled "Lowered Oxygen Tension and EDTA Improve Bovine Zygote Development In Chemically Defined Medium", J. Anim. Sci. (1999), or the SOF medium described in the journal article "Successful Culture In-vitro of Sheep and Cattle Ova", J. Reprod. Fertil. 30:493-497 (1972), each journal article hereby incorporated by reference.

After separating or sorting, sperm cells can be washed by centrifugation for about 10 min at 400 g in collection medium (typically Hepes-tyrode albumin lactate pyruvate medium supplemented with 2.0% bovine serum albumin) followed by suspension in the fertilization medium. Thawed, sorted sperm can be prepared by being centrifuged for 20 minutes at 700 g through a Percoll gradient (90%: 45%) for separation of live and dead sperm. The sperm pellet can then be washed with fertilization medium by centrifugation at 400 g for 10 minutes. Sperm can then be added to to the fertilization medium to give a concentration of 1-2 million/mL.

Table 1. Cleavage Stage of Oocytes Inseminated with Separated Sperm in Four Different Fertilization Media.

Media	No. oocytes	% cleavage	% 2-cell at 24 h	%8-cell at 72 h
Fert-TALP	168	76	6[a]	66
Fert-TALP + neaa	176	71	26[b]	67
CDM	167	89	75[c]	70
SOF	145	86	49[d]	69

[a,b,c,d] Means with different superscripts differ (P<.05).

Now referring primarily to Table 1, as can be understood, oocytes inseminated with separated spermatozoa in fertilization medium containing non-essential amino acids according to the invention exhibit an increased rate of early development through at least the two cell stage.

Table 2. Embryonic Development and Blastocyst Quality Resulting From Fertilization in Four Different Fertilization Media (averaged over two culture media)

Media	No. oocytes	% blastocysts/oocyte		% Grade 1 blastocysts/ total blastocysts
		Total	D7	
Fert-TALP	326	20	17	52[a,c]
Fert-TALP -aa	221	20	17	68[b]
CDM	332	22	18	61[b,c]
SOF	321	21	17	64[b,c]

[a,b,c] Percentages without common superscripts differ (P<.05)

[d] Grade 1 indicates blastocysts with a distinct inner cell mass suitable for embryo transfer.

Now referring primarily to Table 2, some embodiments of the invention in which oocytes are fertilized with sorted spermatozoa in fertilization medium containing supplemented non-essential amino acids can exhibit an enhanced quality of embryos. In embodiments of the invention in which oocytes were fertilized in tyrode albumin lactaate pyruvate (TALP) supplemented with non-essential amino acids derived from Eagles Medium, and further containing 0.6% bovine serum albumin, 20 μg heparin/ mL and 5 mM caffeine there was a difference (P<.05) in quality of embryos as compared to TALP without non-essential amino acids.

Presumptive zygotes can be removed from culture and placed in chemically-defined medium (CDM-1) as discussed in the Journal Animal Science, 78, 152-157 (2000), hereby incorporated by reference, for 6-7 hours after insemination and cultured for 65-66 hours. Embryos that cleaved were further cultured 96 hours in CDM-2 (further

containing MEM essential and non-essential amino acids and 2.0 mM fructose) containing 0.12 IU insulin/mL. Blastocysts were morphologically graded according to the size of inner cell mass and stained with Giemsa to determine cell numbers on day 7 after insemination.

Now referring primarily to Figure 4, the invention further involves a portable incubation system. Certain embodiments of the invention can comprise a straw (19) having an interior volume between about 0.1 mL and about 0.5 mL into which fertilization medium, and oocytes collected from a female mammal, can be transferred. While the straw (19) could be made of any material compatible with the fertilization medium and the collected oocytes, specific embodiments of the straw (19) can be made of plastic, such as or similar to an artificial insemination straw. The ends of plastic straws can be heat sealed after the fertilization medium and the oocytes are transferred inside.

The invention can further comprise an incubation element (20) configured to encapsulate the straw (19) or a plurality of straws inserted within. In some embodiments of the invention the incubation element (20) can be a glass tube having a single sealable aperture element. The aperture element (21) can be sealed with a cap (22), and in some embodiments the cap (22) and the tube can have spiral threads (23) that can be rotationally mated to close the incubation element (20).

After transfer of a straw (19) or a plurality of straws to the interior volume of the incubation element (20), incubation conditions can be established within. Typical incubation conditions within the interior volume of the incubation element can comprise an atmosphere of five percent carbon dioxide in air and a temperature of about 39°C (37 °C to 41 °C). Once incubation conditions are established within the incubation element, the incubation element (20) can be sealed and the oocytes can then be transported within the incubation element (20).

In some embodiments of the invention, oocytes can be transported to a sperm cell separation facility where the incubation element (20) is unsealed, the straw (19) is unsealed and a plurality of sperm cells (15) from a population separated on the basis of

bearing an X-chromosome or bearing a Y-chromosome can be transferred into the straw (19) containing the oocytes. With respect to some embodiments of the invention a concentration of separated sperm cells (15) can be established of between about 1 million to about 2 million/ mL of the fertilization medium. The straw (19) containing the oocytes and spermatozoa in fertilization medium can then be resealed and transferred back into the incubation element (20). The incubation conditions can be re-established and the incubation element sealed. The incubation element (20) containing a straw or plurality of straws (19) can then be transported. During transport the oocytes can become fertilized. Upon arrival zygotes can be transferred from the straw for further culture.

With respect to certain embodiments of the invention, oocytes can first be inseminated with separated or unseparated spermatozoa in conventional 50μl drops and loaded into a 0.25 mL straw or straws (19) within two hours after insemination. Straws (19) can be heat sealed and put into the incubation element (20). The open incubation element containing straws with inseminated oocytes can be equilabrated with 5% carbon dioxide in air at about 39°C for at least one hour and then tightly capped and cultured under the same conditions for between about 18-20 hours.

Again referring primarily to Table 2, fewer oocytes ($P<0.05$) fertilized in Fert-TALP developed to the 2-cell stage by 24 hours than in any other media. Notably, the vast majority of oocytes (75%) fertilized in CDM medium cleaved to 2-cell stabe by this time. By 72 hours post-insemination, there was no difference between any of the media, possibly due to the long 8-cell stage cell cycle.

There was no difference between any of the media on rate of development to blastocysts. However, there was a significant difference in quality of embryos between Fert-TALP and Fert-TALP + non-essential amino acids.

Progression of early bovine embryonic development using separated sperm are similar to studies with in-vivo or in-vitro cleavage of oocytes fertilized with unseparated spermatozoa. In the cow the first in-vivo cleavage occurs at 24-28 hours following ovulation, and the first in-vitro cleavage tages place at 24-48 hours after insemination.

Earlier cleavage occurred with oocytes fertilized in CDM, SOF, and Fert-TALP + aa medium than in conventional Fert-TALP medium. This can be because CDM, SOF, and Fert-TALP + non-essential amino acids, all contain non-essential amino acids, which may play a role in how quickly spermatozoa penetrate oocytes, of in the length of the first cell cycle.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves the staining of spermatozoa, whether fresh spermatozoa or frozen-thawed spermatozoa, separation and isolation techniques which may be used with such stained spermatozoa, as well as devices to accomplish the staining, separation, isolation of such stained spermatozoa into X-chromosome bearing and Y-chromosome bearing populations, and the tramsportion of maturing oocytes and fertilized oocytes. In this patent application, the staining and separating techniques used with spermatozoa are disclosed as part of the results shown to be achieved by the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this international Patent Cooperation Treaty patent application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in functionally-oriented terminology, each aspect of the function is accomplished by a device, subroutine, or program. Apparatus claims may not only be included for the devices described, but also method or process claims may be included to address the

functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims which now be included.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "sorter" should be understood to encompass disclosure of the act of "sorting" -- whether explicitly discussed or not -- and, conversely, were there only disclosure of the act of "sorting", such a disclosure should be understood to encompass disclosure of a "sorter" and even a "means for sorting". Such changes and alternative terms are to be understood to be explicitly included in the description.

Additionally, the various combinations and permutations of all elements or applications can be created and presented. All can be done to optimize the design or performance in a specific application.

Any acts of law, statutes, regulations, or rules mentioned in this application for patent: or patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. Specifically, United States Provisional Patent Application No. 60/253,787, filed November 29, 2000 and United States Provisional Patent Application No. 60/253,785, filed November 29, 2000, are hereby

incorporated by reference including any figures or attachments, and each of references in the following table of references are hereby incorporated by reference.

US Patent Documents

DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	FILING DATE
32,350	02/10/87	Bhattacharya			11/22/74
3,687,806	08/29/72	Van den Bovenkamp	195	1.3	11/04/69
3,829,216	08/13/74	Persidsky	356	36	10/02/72
3,894,529	07/15/75	Shrimpton	128	1 R	04/10/69
4,009,260	02/22/77	Ericsson	424	105	12/11/74
4,067,965	01/10/78	Bhattacharya	424	105	12/17/75
4,083,957	04/11/78	Lang	424	78	02/04/76
4,085,205	04/18/78	Hancock	424	105	01/24/77
4,092,229	05/30/78	Bhattacharya	204	180 R	10/20/76
4,155,831	05/22/79	Bhattacharya	207	299 R	02/23/78
4,191,749	03/04/80	Bryant	424	105	10/11/77
4,225,405	09/30/80	Lawson	204	180 R	08/16/78
4,276,139	06/30/81	Lawson	204	180 R	10/09/79
4,339,434	07/13/82	Ericsson	424	105	08/17/81
4,362,246	12/07/82	Adair	209	3.3	07/14/80
4,448,767	05/15/84	Bryant	424	85	02/15/80
4,474,875	10/02/84	Shrimpton	435	002	08/18/80
4,501,366	02/26/85	Thompson	209	556	12/14/82
4,511,661	04/16/85	Goldberg	436	503	12/30/83
4,605,558	08/12/86	Shrimpton	424	561	04/20/84
4,660,971	04/28/87	Sage et al.	356	39	05/03/84
4,680,258	07/14/87	Hammerling et al	435	7	08/09/83
4,673,288	06/16/87	Thomas et al.			
4,683,195	07/28/97	Mullis et al			
4,683,202	07/28/87	Mullis			
4,698,142	10/06/87	Muroi et al	204	182.3	07/31/85
4,749,458	06/07/88	Muroi et al	204	182.3	03/02/87
4,790,653	12/13/88	North, Jr.			
4,988,619	01/29/91	Pinkel	435	30	11/30/87
4,999,283	03/12/91	Zavos et al	435	2	08/18/89
5,021,244	06/04/91	Spaulding	424	561	05/12/89
5,055,393	10/08/91	Kwoh et al			
5,135,759	08/04/92	Johnson	424	561	04/26/91
5,346,990	09/13/94	Spaulding	530	350	03/12/91
5,371,585	12/06/94	Morgan et al.	356	246	11/10/92
5,437,987	08/01/95	Ten et al			
5,439,362	08/08/95	Spaulding	424	185.1	07/25/94
5,461,145	10/24/95	Kudo et al			
5,466,572	11/14/95	Sasaki et al.	435	2	04/25/94
5,480,774					
5,483,469	01/09/96	Van den Engh et al.	364	555	08/02/93
5,494,795	2/27/96	Guerry et al.	435	6	5/5/93
5,503,994	04/02/96	Shear et al.	436	90	10/08/93
5,578,449	11/26/96	Frasch et al.	435	6	4/20/95
5,514,537	05/07/96	Chandler	435	002	11/28/94

5,589,457	12/31/96	Wiltbank	514	12	07-03-95
5,602,039	02/11/97	Van den Engh	436	164	10/14/94
5,602,349	02/11/97	Van den Engh	73	864.85	10/14/94
5,622,820	4/11/97	Rossi	435	5	11/3/94
5,641,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,643,796	07/01/97	Van den Engh et al	436	50	10/14/4
5,660,997	08/26/97	Spaulding	435	7.21	06/07/95
5,690,895	11/25/97	Matsumoto et al.	422	73	12/06/96
5,700,692	12/23/97	Sweet	436	50	09/27/94
5,726,364	03/10/98	Van den Engh	73	864.85	02/10/97
5,819,948	10/13/98	Van den Engh	209	158	08/21/97
5,876,942	3/2/99	Cheng et al	435	6	7/24/97
5,880,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,985,216	11/16/99	Rens, et al.	422	073	07/24/97
6,071,689	06/06/00	Seidel et al.	435	2	01/29/98

Foreign Documents

DOCUMENT NO	DATE	COUNTRY
WO 96/12171	10/13/95	United States
WO 98/34094	06/08/98	NZ
WO 99/05504	07/24/98	US
WO 99/33956	08/07/99	US
WO 99/38883	05/08/99	US
WO 99/42810	08/26/99	US
WO 00/06193	10/02/00	US

Other Reference Documents

Roser, JF., Evans, J.W., Kiefer, DP., Neeley, D.P. and Pacheco, C.A. 1980. Reproductive efficiency in mares with anti-hCG antibodies. Proc 9th Int. Congr. Artira. Repro. and A.I. 4:627. abstr.
"Applying Semen Sexing Technology to the AI Industry", National Association of Animal Breeders, September 2000, pp. 1-16
"Sexed Semen Offers Faster Genetic Gain", Farming News, Livestock Supplement, February 1997, p. 28.
Akhtar, S., et al., "Prevalence of Five Stereotypes of Bluetongue Virus in a Rambouillet Sheep Flock in Pakistan", Veterinary ecord 136, 1995, p. 495.
Akhtar, S., et al., "Sex Preselected in Cattle: a Field Trial", Veterinary Record 136, 1995, p. 495-496.
Aldrich, S. L., Berger, L.L., Reiling, B.A., Kegler, D.I., and Nagh, T.G.. 1995. "Parturition and periparturient reproductive and metabolic hormone concentration in prenatally androgenized beefheifer", I. Anim. Sci. 73:3712.
Amann, R. P. "Issues affecting commercialization of sexed sperm". Therio: 52:1441, 1999
Amann, R.P. et al, "Prospects For Sexing Mammalian Sperm," Colorado Associated University Press, Animal Reproduction Laboratory College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523, 1982
American Meat Science Association in cooperation with National Livestock and Meat Board. "Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meatK", 1995
Amoah, E.A. and Gelaye, S. 1996. Biotechnological advances in goat reproduction. J. Anim. Sci. 75(2):578-585.
Andersen, V.K., Aamdal, J. and Fougner, J.A. 1973. Intrauterine und tiefzervikale Insemination mit Gefriersperma bein Schat. Zuchthygiene. 8:113-118.
Bagley, C. P. 1993. Nutritional management of replacement beef heifers -A review. J. Anim. Sci. 71:3155-3163.
Bailey, C. M., Reid, C.R., Ringkob, T.P., Koh, Y.O., and Foote, W.D. "Nulliparous versus primiparous crossbred females for beef." J. Anim. Sci. 69:1403., 1991

Baker, R.D., Dziuk, P.J. and Norton, H.W. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci. 27:88-93.
Barnes, F.L.. and Eyestone, W.H., "Early Cleavage and the Maternal Zygotic Transition in Bovine Embryos", Theriogeneology, Vol. 33, No. 1, January 1990, pp. 141-149
Becker, S.E. and Johnson, A.L. 1992. Effects of gonadotropin releasing hormone infused in a pulsatite or continuous fashion on serum gonadotropin concentrations and ovulation in the mare. J. Anim. Sci. 70:1208-1215.
Bedford, S .J. and Hinrichs, K. 1994. The effect of insemination volume on pregnancy rates of pony mares. Theriogenology 42:571-578.
Bellows, R. A., Short, R.E., Anderson, D.C., Knapp, B.W., and Pahnish, O.F. "Cause and effect relationships associated with calving difficulty and calfbirth weight", J. Anim. Sci. 33:407, 1971
Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K.lnskeep. "Sourceof progesterolle prior to puberty in beef heifers". J. Anim. Sci. 49:1276., 1979
Berger, G.S. 1987. Intratubal insemination. Fert. Steril. 48:328-330.
Bergfeld, E. G., Kojima, F.N., Cupp, A.S., Wehnnan, M.E., Peters, K.T., Garciawinder, M., and Kinder, J.E., "Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy-intake", Bio. of Repro. 51:1051, 1994
Berry, B. W.,Smith, G.C., and Carpente.zl, "Beef carcass maturity indicators and palatability attributes", J. Anim. Sci. 38:507, 1974
Beyhan, Z., et al., "Sexual Dimorphism in IVF Bovine Embryos Produced by Sperm Sorted by High Speed Flow Cytometry", Theriogenology 49, 1998, p. 359.
Blanchard, T. and Dickson, V., "Stallion Management", The Veterinary Clinics of North America, Equine Practice, Vol. 8, No. 1, April 1992, pp 207 - 218.
Bond, J., et al., "Growth and carcass traits of open beef heifers versus beef heifers that have calved", Nutrition Reports International 34:621. 1986
Boucque, C. V., et al., "Beef-production with maiden and once-calved heifers", Livestock Prod. Sci. 7:121. 1980
Bourdon, R. M. and J. S. Brinks. "Simulated efficiency of range beef -production". Culling strategies and nontraditional management-systems. J. Anim. Sci.65:963. 1987
Bracher, V. and Allen, W.R., "Videoendoscopic Examination of the Mare's Uterus: Findings in Normal Fertile Mares", Equine Veterinary Journal, Vol. 24 (1992), pp. 274-278
Braselton, W.E. and McShan, W.H. 1970. "Purification and properties of follicle stimulating and luteinizing hormones from horse pituitary glands", Arch. Biochem. Biophys. 139:45-48.
Brethour, J. R., "The single-calfheifer system", Kans. Agric. Sta. Rep. Frog. 570. 1989
Bristol, S.P. 1982. Breeding behavior of a stallion at pasture with 20 mares in synchronized oestrus. J. Reprod. Fert. Suppl. 32:71.
Brookes, A. J. and Obyme, M., "Use of cow-heifers in beef production", J. of the Royal Agricultural Society of England 126:30. 1965
Buchanan, B.R., et al, "Insemination of Mares with Low Numbers of Either Unsexed or Sexed Spermatozoa", Theriogenology, Vol. 53, pp 1333-1344, (2000)
Burns, P. D. and Spitzer, J.C., "Influence ofbiostimulation on reproduction in postpartum beef-cows", J. Anim. Sci. 70:358. 1992
Burwash, L.D., Pickett, B.W., Voss, J.L. and Back, D.G. 1974. "Relatioship of duration of estms to pregnancy rate in normally cycling, non-lactating mares" J.A.V.M.A. 165:714-716.
Byerley, D. J., et al., "Pregnancy rates of beef heifers bred either on puberal or 3rd estrus". J Anim. Sci. 65:645. 1987
Caslick, E.A., "The Vulva and the Vulvo-vaginal Orifice and its Relation to Genital Health of the Thoroughbred Mare", Cornell Veterinarian, Vol. 27, 1937, pp. 178-187
Catt, et al., "Assessment of Ram and Boar Spermatozoa During Cell-Sorting by Flow Cytometry", Reproduction Dom Animal, Vol. 32, 1997, pp 251-258.
Catt, S.L., et al., "Birth of a Male Lamb Derived from an In Vitro Matured Oocyte Fertilized by Intracytoplasmic Injection of a Single Presumptive Male Sperm", Veterinary Record 139, 1996, pp. 494-495.
Chin, W.W. and Boime, I. 1990. *In:* Glycoprotein Hormones. Serona Symp. Norwell, MA. pp. 19-20
Chung, Y.G., Schenk, J.L., Herickhoff, L.A. and Seidel, G.E. Jr. 1998. Artificial insemination of superovulated heifers with 600,000 sexed sperm. J Anim. Sci. Suppl. 1. 836:215. abstr.
Clement, F., Vincent, P., Mahla, R., Meriaux, J.C. and Palmer, E. 1998. Which insemination fertilizes when

several successive inseminations are performed before ovulation. 7th Int. Symp. Eq. Repro. 151. abstr.
Coleou, J., et al., "Essai de velage tres precoce de genisses en vue de la production de viande." Essai Vauz/ Aure no.50, programme USFGC-INAPG-ITFC. 1974
Cran, D.G., et al., "Production of Bovine Calves Following Separation of X- and Y- Chromosome Bearing Sperm and In Vitro Fertilisation", Veterinary Record 132, 1993, pp. 40-41.
Cran, D.G., et al., "Production of Lambs by Low Dose Intrauterine Insemination with Flow Cytometrically Sorted and Unsorted Semen", Theriogenology 47, 1997, p. 267.
Crowley, J. P. The facts of once-bred heifer production. (Ed) J.B. Owens. The maiden female-a means of increasing meat production. School of Agric., Univ. of Aberdeen, Scotland. 1973
Curran, S. 1998. In: Equine Diagnostic Ultrasonography. Fetal gender determination. Rantanen & McKinnon. 1st Ed. Williams and Wilkins. pp. 165-169.
Day, B.N., Abeydeera, L.R., Johnson, L.A., Welch, G.R., Wang, W.H., Cantley, T.C. and Rieke, A. 1998. Birth of piglets preselected for gender following in vitro fertilization of in vitro matured pig oocytes by X and Y bearing spermatozoa sorted by high speed flow cytometry. Theriogenology. 49(1):360. abstr.
Dean, P.N., Pinkel, D. and Mendelsob. n, M.L. 1978. Hydrodynamic orientation of spermatozoa heads for flow cytometry. Biophys. J. 23:7-13.
Demick, D.S., Voss, J.L. and Pickett, B.W. 1976. Effect of cooling, storage, glycerization and spermatozoal numbers on equine fertility. J. Anim. Sci. 43:633-637.
DenDaas, J.H.G., De Jong, G., Lansbergen, L.M.T.E. and Van Wagtendonk-De Leeuw, A.M. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of dairy bulls. J Dairy Sci. 81: 1714-1723.
Denham, A. "In-vitro studies on sandhill range forage as related to cattle preference", M.S. Thesis. 1965. Colorado State University.
Deutscher, G. H. "Extending interval from seventeen to nineteen days in the melengestrol acetate-prostaglandin estrous synchronization program for heifers". The Professional Animal Scientist 16:164. 2000
"Diagnostic Products Corporation. Coat-A-Count", Progesterone.com. 1998.
Dikeman, M. E. "Cattle production systems to meet future consumer demands. J. Anim. Sci. 59:1631, 1984
Dinnyes, A., et al., "Timing of the First Cleavage Post-insemination Affects Cryosurvival of In Vitro-produced Bovine Blastocysts", Molec Reprod Develop 53, 1999, pp 318-324.
Donaldson, L. E., "Effect of Insemination Regimen on Embryo Production in Superovulated Cows", The Veterinary Record, July 13, 1985, pp. 35-37
Donoghue, A.M., Byers, A.P., Johnston, L.A., Armstrong, D.L. and Wildt, D.E. 1996. Timing of ovulation after gonadotropin induction and its importance to successful intrauterine insemination in the tiger (Panthera tigris). J. Reprod. Fert. 107:53-58.
Douglas, R.H. 1979. Review of superovulation and embryo transfer in the equine. Theriogenology. 11:33-46.
Douglas, R.H., Nuti, L. and Ginther, O.J. 1974. Induction of ovulation and multiple ovulation on seasonally-anovulatory mares with equine pituitary fractions. Theriogenology. 2(6): 133-142.
Doyle, S. P., et al. "Artificial insemination of lactating angus cows with sexed semen". Proc. Western Sect. Am.Soc.Anim. Sci. 50:203. 1999
Duchamp, G., Bour, B., Combamous, Y. and Palmer, E. 1987. Alternative solutions to hCG induction of ovulation in the mare. J. Reprod. Fert. Suppl. 35:221-228.
Evans, M.J. and Irvine, C.H.G. 1977. Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares. Bio. Reprod. 16:452-462.
Ferrell, C. L. and T. G. Jenkins. "Energy-Utilization by Mature, nonpregnant, nonlactating cows of different types" J. Anim. Sci. 58:234. 1984
Ferrell, C. L. "Effects ofpost-weaning rate of gain on onset of puberty and productive performance of heifers of different breeds. J. Anim. Sci. 55:1272. 1982
Field, R. A., et al., "Bone-ossification and carcass characteristics ofwethers given silastic implants containing estradiol". I. Anim. Sci. 68:3663-3668. 1990
Field, R., R. et al., "Growth, carcass, and tenderness characteristics ofvirgin, spayed, and single-calfheifers.", J. Anim. Sci. 74:2178. 1996
Fitzgerald, B.P., Peterson, K.D. and Silvia, P.J. 1993. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: Preliminary evidence on suppression of ovulation during the breeding season. Am. J. Vet. Res. 54:1746-1751.

Fluharty, F. L., et al., "Effect of weaning and diet on growth of calves." Research and Reviews. The Ohio State University Department of Animal Sciences. 1996
Fluharty, F.L., et al., "Effects of Age at Weaning and Diet on Growth of Calves", Ohio Agri. Res. and Dev. Circular, 1996, 156: 29.
Foulkes, J.A., Stewart, D.L. and Herbert, C.N. 1977. Artificial insemination of cattle using varying numbers of spermatozoa. Vet. Rec. 101:205.
Fugger, E.F., "Clinical Experience with Flow Cytometric Separation of Human X- and Y- Chromosome Bearing Sperm", Theriogenology, Vol. 52, pp. 1435-1440 (1999)
Fulwyler, M.J. 1965. Electronic separation of biological cells by volume. Science. 150:910.
Fulwyler, M.J. 1977. Hydrodynamic orientation of cells. J Histochem. Cytochem. 25:781-783.
Seidel, G.E.. Jr., "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Diary, Loveland, CO, USA January 1996.
Garner, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D., Van Dilla, M.A. and Johnson, L.A. 1983. "Quantification of the X and Y chromosome-bearing spermatozoa of domestic animals by flow cytometry". Biol. Reprod. 28:312-321.
Ginther, O.J. 1983. Sexual behavior following introduction of a stallion into a group of mares. Theriogenology. 19:877.
Ginther, O.J. 1992. In: Reproductive Biology of the Mare. (2nd Ed.) Equiservices, Cross Plains, WI.
Gledhill, B.L. 1988. Gender preselection: historical, technical and ethical perspective. Semin Reprod. Endocrinol. 6:385-395.
Gombe, S. and Hansel, W. "Plasma luteinizing-hormone (LH) and progesterone levels in heifers on restricted energy intakes." J. Anim. Sci. 37:728. 1973
Gourley, D.D. and Riese, R.L. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. N. Amer: Food Anim. Prac. 6(3):615-633.
Gravert, H. 0., "Genetic Aspects of Early Calving." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 59. 1975
Gregory, K. E., et al., "Characterization of biological types of cattle III .2." Growth-rate and puberty in females. J. Anim. Sci. 49:461. 1979
Grimes, I. F, and T. B. Turner. "Early weaning of fall born calves II." Post weaning performance of early and normal-weaned calves. I. Prod. Agric. 4:168. 1991
Grondahl, C., et al, "In Vitro Production of Equine Embryos", Biology of Reproduction, Monograph Series I, pp. 299-307 (1995)
Guillou, F. and Combamous, Y. 1983. Purification of equine gonadotropins and comparative study of their acid-dissociation and receptor-binding specificity. Biochem. Biophys. Acta. 755:229-236.
Gurnsey, M.P., and Johnson, L.A., "Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bering sperm of domestic animals: a review", 1998, New Zealand Society of Animal Protection, three pages.
Hall, J. B., et al., "Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers." J. Anim. Sci. 75:1606. 1997
Hamano, K., et al., "Gender Preselection in Cattle with Intracytoplasmically Injected, Flow Cytometrically Sorted Sperm Heads", biology of Reproduction 60, 1999, pp. 1194-1197.
Harrison, L.A., Squires, E.L. and McKinnon, A.O. 1991. Comparison of hCG, buserelin and luprostiol for induction of ovulation in cycling mares. Eq. Vet. Sci. 3:163-166.
Harte, F. J. "System of production of bee from once calved heifers." In: J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 123. 1975
Hawk, H.W., et al., "Fertilization Rates in Superovulating Cows After Deposition of Semen on the Infundibulum Near the Uterotubal Junction or After Insemination with High Numbers of Sperm", XP-002103478, Theriogenology, May 1988, Vol. 29, No. 5, pp 1131-1142.
Hemlesmeyer, G. N., et al. "Effects of lactation and prenatal androgenization on the perfomlance, carcass coompostion and longissimus muscle sensory characteristics ofheifers in the single-calfheifer system." The Professional Animal Scientist 15:14. 1999
Hennegmeyer, G. N., et al. "Effects of prenatal androgenization and implantation on the performance and carcass

composition of lactating heifers in the single-calfheifer system." The Professional Animal Scientist 15:173. 1999

Hilton, G. G., et al., "An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows." I. Anim. Sci. 76:2094. 1998

Ho, L., et al., "Influence of gender, breed and age on maturity characteristics of sheep." J. Anim. Sci. 67:2460-2470. 1989

Hofferer, S., Lecompte, F., Magallon, T., Palmer, E. and Combamous, Y. 1993. Induction of ovulation and superovulation in mares using equine LH and FSH separated by hydrophobic interaction chromatography. J. Reprod. Fert. 98:597-602.

Hohenboken, W. D. "Applications of sexed semen in cattle production." Therio.52:1421. 1999

Holtan, D.W., Douglas, R.H. and Ginther, O.J. 1977. Estrus, ovulation and conception following synchronization with progesterone, prostaglandin F2 ct and human chorionic gonadotropin in pony mares. J. Anim. Sci. 44:431-437.

Householder, D.D., Pickett, B.W., Voss, J.L. and Olar, T.T. 1981. Effect of extender, number of spermatozoa and hCG on equine fertility. J. Equine Vet. Sci. 1:9-13.

Howard, J.G., Bush, M., Morton, C., Morton, F., Wentzel, K. and Wildt, D.E. 1991. Comparative semen cryopreservation in ferrets (Mustela putorious furo) and pregnancies after laparoscopic intrauterine insemination with frozen-thawed spermatozoa. J. Reprod. Fert. 92:109-118.

Howard, J.G., Roth, T.L., Byers, A.P., Swanson, W.F. and Wildt, D.E. 1997. Sensivity to exogenous gonadotropins for ovulation and laparoscopic artificial insemination in the theetab and clouded leopard. Biol. Reprod. 56:1059-1068.

Hunter, R.H.F. 1980. Transport and storage of spermatozoa in the female reproductive tract. Proc 4th Int. Congr. Artira. Repro. and A.I. 9:227-233.

Hyland, J.H., Ainsworth, C.G.V. and Langsford, D.A. 1988. Gonadotropin-releasing hormone (GnRH) delivered by continuous infusion induces fertile estrus in mares during seasonal acyclicity. Proc. Amer. Assoc. Eq. Prac. 181-190.

Irvine, C.H.G. and Alexander, S.L. 1993. *In:* Equine Reproduction. Edited by McKirmon and Voss. Lea and Febiger. Philadelphia, London. pp. 37.

Jafar, et al., "Sex Selection in Mammals: A Review", Theriogenology, vol. 46, 1996, pp 191-200.

Jarriage, R. "Age of cows at first calving in France." J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 10. 1975

Jasko, D.J., Martin, J.M. and Squires, E.L. 1992. Effect of volume and concentration of spermatozoa on embryo recovery in mares. Theriogenology. 37:1233-1239

Johnson L.A., et al., 1987. Flow cytometry of X- and Y- chromosome bearing sperm for DNA using an improved preparation method and staining with Hoechst 333-42. Garnete Research 17: 203-212

Johnson, "Gender preselection in Mammals: An overview", Dtsch. Tierarztl. Wschr, Vol. 103, Aug./Sep. 1996, pp 288-291.

Johnson, A.L. 1986. Pulsatile release of gonadotropin releasing hormone advances ovulation in cycling mares. B iol. Reprod. 35:1123E 1130.

Johnson, A.L. and Becker, S.E. 1988. Use of gonadotropin-releasing hormone (GnRH) treatment to induce multiple ovulations in the anestrous mare. Eq. Vet. Sci. 8:130-134.

Johnson, L., "Sex Preselection by Flow Cytometric Separation of X and Y Chromosome-Bearing Sperm Based on DNA Difference: a Review", Reproduction and Fertilization Development 7, 1995, pp. 893-903.

Johnson, L., "Successful Gender Preselection in Farm Animals", Agricultural Biotechnology, 1998, pp. 439-452.

Johnson, L.A. 1988. Flow cytometric determination of spermatozoa sex ratio in semen purportedly enriched for X or Y bearing spermatozoa. Theriogenology. 29:265. abstr.

Johnson, L.A. 1992. Gender preselection in domestic animals using flow cytometrically sorted sperm. J Anim. Sci. Suppl 1.70:8-18.

Johnson, L.A. 1994. Isolation of X- and Y-bearing spermatozoa for sex preselection. *In:* Oxford Reviews of Reproductive Biology. Ed. HH Charlton. Oxford University Press. 303-326.

Johnson, L.A. 1995. Sex preselection by flow cytometric separation of X and Y chromosome bearing spermatozoa based on DNA difference: a review. Reprod. Fert. Dev. 7:893-903.

Johnson, L.A. and Schulman, J.D. 1994. The safety of sperm selection by flow cytometry. Ham. Reprod. 9(5):758.

Johnson, L.A., "Sex preselection in swine: altered sex ratios in offspring following surgical insemination of flow-

sorted X- and Y-bearing sperm", Reprod. Domest. Anim. 26:309-314, 1991
Johnson, L.A., and Pinkel, D., "Modification of a Laser-Based flow Cytometer for High-Resolution DNA Analysis of Mammalian Spermatozoa", Cytometry 7, 1986, pp 268 - 273.
Johnson, L.A., et al., "Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting", Exceptional Paper-Rapid Publication, XP-002103476, Biology of Reproduction 41, 199-203, 1989, pp 199-203.
Johnson, L.A., et al., 1994. Improved flow sorting resolution of X- and Y- chromosome bering viable sperm separation using dual staining and dead cell gating. Cytometry 17 (suppl 7):83.
Johnson, L.A., Flook, J.P., Look, M.V. and Pinkel, D. 1987b. Flow sorting of X and Y chromosome bearing spermatozoa into two populations. Gam. Res. 16:203-212.
Johnson, L.A., Welch, G.R., Rens, W. and Dobrinsky, J.R. 1998. Enhanced flow cytometric sorting of manunalian X and Ysperm: high speed sorting and orienting no77.1e for artificial insemination. Theriogenology. 49(1):361. abstr.
Joseph, R. L. "Carcass composition and meat quality in once calved heifers." In: J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 143. 1975
Joseph, R. L. and J. P. Crowley. "Meat quality of once-calved heifers." Irish J. of Agric. Research 10:281. 1971
Kachel, V., et al., AUniform Lateral Orientation, Cused by Flow Forces, of Flat Particles in Flow-Through Systems@, The Journal of Histochemistry and Cytochemistry, 1997, Vol. 25, No. 7, pp 774 -780.
Kanayama, K., Sankai, T., Nariaik, K., Endo, T. and Sakuma, Y. 1992b. Pregnancy by means of tubal insemination and subsequent spontaneous pregnancy in rabbits. J. Int. Med. Res. 20:401-405.
Karabinus, et al., "Effects of Egg Yolk-Citrate and Milk Extenders on Chromatin Structured Viability of Cryopreserved Bull Sperm", Journal of Dairy Science, Vol. 74, No. 11, 1999, pp 3836-3848.
Keeling, P. C. B. M. S. T. G. D. I. a. P. W. J., "A modeling study of once-bred heifer beef production." Proceedings of the New Zealand Society of Animal Production. 51. 1991
Kilicarslan, M.R., Horoz, H., Senunver, S.C., Konuk, S.C., Tek, C. and Carioglu, B. 1996. Effect of GnRH and hCG on ovulation and pregnancy in mares. Vet. Rec. 139:119-120.
Kinder, J. E., et al. "Endocrine basis for puberty in heifers and ewes." J. Repro. and Fertility 393. 1995
Klindt, J. and J. D. Crouse. "Effect of ovariectomy and ovariectomy with ovarian auto transplantation on feedlot performance and carcass characteristics ofheifers." J. Anim. Sci. 68:3481. 1990
Klosterman, E. W. and C. F. Parker. "Effect of size, beed and sex upon feed efficiency in beef cattle." North Central Regional Research Publication 235, Ohio Agric. Research and Development Center 1090:3. 1976
Kniffen, D. M., Wagner, W.R., and Lewis. P.E. "Effects oflong-tenn estrogen implants in beef heifers." I. Anim. Sci. 77:2886. 1999
Koch, R. M., et al., "Characterization of biological types of cattle -Cycle-II .3." Carcass composition, quality and palatability. I. Anim. Sci. 49:448. 1919
Lapin, D.R. and Ginther, O.J. 1977. Induction of ovulation and multiple ovulations in seasonally anovulatory and ovulatory mares with an equine pituitary extract. J. Anim. Sci. 44:834-842.
Laster, D. B., "Factors affecting dystocia and effects of dystocia on subsequent reproduction in beef-cattle." J. Anim. Sci. 36:695. 1973
Lawrenz, R. 1985. Preliminary results of non-surgical intrauterine insemination of sheep with thawed frozen semen. J S Afr. Vet. Assoc. 56(2):61-63.
Levinson, G., et al, 1995. DNA-based X-enriched sperm separation as an adjunct to preimplantation genetic testing for the preparation of X-linked disease. Mol. Human Reprod. 10:979-982.
Lindsey, A., et al., AHysteroscopic Insemination of Mares with Nonfrozen Low-dose Unsexed or Sex-sorted Spermatozoa@, currently unpublished, pp. 1-15.
Linge, F. 1972. Faltforsok med djupfrost sperma (field trials with frozen sperm). Farskotsel. 52:12-13.
Lonergan, P., et al., "Effect of Time Interval from Insemination to First Cleavage on the Development of Bovine Embryos In Vitro and In Vivo", Theriogenology, 1999, p. 326
Long, C.R., Rath, D., Welch, G.R., Schreier, L.L., Dobrinsky, J.R. and Johnson, L.A. *1998.* AIn vitro production of porcine embryos from semen sorted for sex with a high speed cell sorter: comparison of two fertilization media.@, Theriogenology. 49(1):363. abstr.
Loy, R.G. and Hughes, J.P. 1965. The effects of human chorionic gonadotropin on ovulation, length of estrus, and fertility in the mare. Cornell Vet. 56:41-50.
Lu, K.H., et al., "In Vitro Fertilization with Flow-Cytometrically-Sorted Bovine Sperm", Theriogenology 52,

1999, pp. 1393-1405.
Lynch, I. M., et al., "Influence of timing of gain on growth and reproductive performance ofbeefreplacement heifers." I. Anim. Sci. 75:1715. 1997
Macmillan, K.L. and A.M. Day, "Prostaglandin F2a - A Fertility Drug In Dairy Cattle?"„ Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand, Theriogenology, September 1982, Vol. 18 No. 3, pages 245-253
Martin, A. H., et al., "Characteristics of youthful beef carcasses in relation to weight, age and sex .3. meat quality attributes." Canadian I. Anim. Sci. 51:305. 1971
Martin, L. C., J. S. Brinks, R. M. Bourdon, and L. V. Cundiff. "Genetic-effects on beef heifer puberty and subsequent reproduction." J. Anim. Sci. 70:4006. 1992
Matsuda, Y. and Tobari, I. 1988. Chromosomal analysis in mouse eggs fertilized *in vitro* with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). Mutat. Res. 198:131-144.
Matulis, R. J., F. K. Mckeith, D. B. Faulkner, L. L. Berger, and P. George. "Growth and carcass characteristics of cull cows after different times-on-feed." J. Anim. Sci. 65:669. 1987
Mauleon, P. "Recent research related to the physiology of puberty." Commission of the European Communities. The early calving of heifers and it's impact on beef production. 1975
Maxwell, W. and Johnson, L., "Chlortetracycline Analysis of Boar Spermatozoa after Incubation, Flow Cytometric Sorting, Cooling, or Cryopreservation", Molecular Reproduction and Development 46, 1997, pp. 408-418.
Maxwell, W.M.C., Evans, G., Rhodes, S.L., Hillard, M.A. and Bindon, B.M. 1993. Fertility of Superovulated Ewes after Intrauterine or Oviductal Insemination with Low Numbers of Fresh or Frozen-Thawed Spermatozoa. Reprod. Fertil. Dev. 5:57-63.
Mccomlick, R. J. "The flexibility of the collagen compartment of muscle." Meat Sci. 36:79. 1994
McCue, P.M. 1996. Superovulation. Vet. Clin. N. Amer. Eq. Prac. 12:1-11.
McCue, P.M., Fleury, J.J., Denniston, D.J., Graham, J.K. and Squires, E.L. 1997. Oviductal insemination in the mare. 7th Int Symp. Eq. Reprod. 133. abstr.
McDonald, L.E. 1988. Hormones of the pituitary gland. *In:* Veterinary Pharmacology and Therapeutics. 6th ed. Edited by N.H. Booth and L.E. McDonald. Ames, Iowa State Univ. Press. pp. 590.
McKenna, T., Lenz, R.W., Fenton, S.E. and Ax, R.L. 1990. Nonreturn rates of dairy cattle following uterine body or comual insemination. J. Dairy Sci. 73:1179-1783.
McKinnin, A. and Voss, J., "Equine Reproduction", Lea & Febiger, Philadelphia, 1993, pp 291, 299 - 302, 345 - 348, 739 - 797.
McKinnon, A. et al, 1993. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. Eq. Vet. J. 25:321-323.
McKinnon, A.O. et al, 1996. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. Eq. Vet. J. 29:153-155.
McNutt, et al., "Flow Cytometric Sorting of Sperm: Influence on Fertilization and Embryo/Fetal Development in the Rabbits", Molecular Reproduction and Development, Vol. 43, 1996, pp 261-267.
Meilgaard, M., G. V. Civille, and B. T. Carr. "Sensor Evaluation Techniques." CRC Press Inc., Boca Raton, FL. 1991
Meinert, C., et al., "Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin", Equine Veterinary Journal, 25, 1993, pp 65 - 68.
Merton, J., et al., "Effect of Flow Cytometrically Sorted Frozen/Thawed Semen on Success Rate of In Vitro Bovine Embryo Production", Theriogenology 47, 1997, pp. 295.
Meyers, P.J., Bowman, T., Blodgett, G., Conboy, H.S., Gimenez, T., Reid, M.P., Taylor, B.C., Thayer, J., Jochle, W. and Trigg, T.E. 1997. Use of the GnRH analogue, deslorelin acetate, in a slow release implant to accelerate ovulation in oestrous mares. Vet. Rec. 140:249-252.
Michaels, Charles, "Beef A.I. Facilities that work", Proc. Fifth N.A.A.B Tech. Conf. A.I. Reprod. Columbia, MO. pp. 20-22.
Michel, T.H., Rossdale, P.D. and Cash, R.S.G. 1986. Efficacy of human chorionic gonadotrophin and gonadatrophin releasing hormone for hastening ovulation in Thoroughbred mares. Eq. Vet. J. 6:438-442.
Miller, S.J. 1986. *Artificial Breeding Techniques in Sheep. In* Morrow, D.A. (ed): Current Therapy in Theriogenology 2. Philadelphia, WB Saunders.
Mirskaja, L.M. and Petrapavlovskii, V.V. 1937. The reproduction of normal duration of heat in the mare by the

administration of Prolan. Probl. Zivotn. Anim. Breed. Abstr. 5:387.
Moe, P. W., H. F. Tyrrell, and W. P. Flatt. "Energetics ofbodytissue mobilization." J. of Dairy Sci. 54:548.
Molinia, F.C., Gibson, R.J., Brown, A.M., Glazier, A.M. and Rodger, J.C. 1998. Successful fertilization after superovulation and laparoscopic intrauterine insemination of the brushtail possum, *Trichosurus vulpecula,* and tammar wallaby, *Macropus eugenii.* J.Reprod. Fert. 112:9-17.
Moms, S. T., et al., "Biological efficiency: How relevent is this concept to beef cows in a mixed livestock seasonal pasture supply context?" Proceedings of the New Zealand Society of Animal Production 54:333. 1994
Monensin." J. Anim. Sci. 55:357-362. 1982
Moran, C., J. F. Quirke, and J. F. Roche. "Puberty in heifers -a review." Animal Reproduction Sci. 18:167. 1989
Morcom, C.B. and Dukelow, W.R. 1980. A research technique for the oviductal insemination of pigs using laparoscopy. Lab. Anim. Sci. 1030-I031.
Morgan, J. B., et al., "National beef tenderness survey." J. Anim. Sci.69:3274. 1991
Morris, L.H., et al., "Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares", Journal of Reproduction and Fertility, Vol. 118, pp. 95-100 (2000)
Moseley, W. M., et al., 1982. "Relationship of Growth and Puberty in Beef Heifers Fed
Mount, D. E. "Fibrous and non-fibrous carbohydrate supplementation to ruminants grazing forage from small grain crops." M.S. Thesis. Colorado State University. 2000
Muller, W. and Gautier, F. 1975. Interactions of heteroaromatic compounds with nucleic acids. Euro. J Biochem. 54:358.
Munne, S. 1994. Flow cytometry separation of X and Y spermatozoa could be detrimental to human embryos. Hum. Reprod. 9(5):758
Myers, S. E., "Performance and carcass traits ofearly-weaned steers receiving either a pasture growing period or a finishing diet at weaning." J. Anim. Sci. 77:311. 1999
Myers, S. E., et al., "Comparison of three weaning ages on cow-calfperformance and steer carcass traits." J. Anim. Sci. 77:323. 1999
Myers, S. E., et al., "Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers." J. Anim. Sci. 77:300. 1999
Nix, I. P., I. C. Spitzer, and P. I. Chenoweth. "Serum testosterone concentration, efficiency of estrus detection and libido expression in androgenized beef cows." Therio. 49: 1195. 1998
Nowshari, et al., "Superovulation of Goats with Purified pFSH Supplemented with Defined Amounts of pLH", Theriogenology, Vol 43, 1995, pp 797-802.
Nowshari, et al., Theriogenology, Vol. 43, 1995, pp 797-802.
NRC. Nutrient requirements for beef cattle. National Academy of Sci. National Research Council, Washington, DC. 1996
Olson, S.E. and Seidel, G.E. Jr., "Reduced Oxygen Tension and EDTA improve Bovine Zygote Development in a Chemically Defined Medium", Journal of Animal Science 78, 2000, pp. 152-157.
Owen, J. B. "The maiden female-a means of increasing meat production." Proc. Symp. on the use of once bred heifers and gilts. 1973
Pace, M.M. and Sullivan, J.J. 1975. Effect of timing of insemination, numbers of spermatozoa and extender components on pregnancy rates in mares inseminated with frozen stallion semen. J Reprod. Fert. Suppl. 23:115-121.
Parent US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.
Parrish, J., et al., "Capacitation of Bovine Sperm by Heparin", Technology of Reproduction 38, 1988, pp. 1171-1180.
PCT application , PCT/US99/17165, filed 28 July 1999, entitled "Equine System for Non-Surgical Artificial Insemination".
PCT application, PCT/US98/27909, filed 31 December 1998, entitled "Commercially Practical Sex-Specific Insemination of Mammals".
Peippo, J., et al., "Sex diagnosis of equine preimplantation embryos using the polymerase chain reaction", Theriogenology, Vol. 44 619-627 (1995)
Perry, E.J. 1968. Historical Background In: *The Artificial Jnsemination of Farm Animals.* 4th ed. Edited by E.J. Perry. New Brunswick, Rutgers University Press, pp. 3-12.
Petersen, G.A., et al, "Cow and Calf Performance and Economic Considerations of Early Weaning of Fall-Born

Beef Calves", J. Anim. Sci., 1987, 64:15, pp 15-22.
Petit, M. "Early Calving in Suckling Herds." In: (Ed.) J.C. Taylor. The early calving of heifers and it's impact on beef production. 157. 1975
Pickett GW, et al., "Management of the mare for maximum reproductive efficiency", Bulletin No. 6 Colorado State University, Ft. Collins CO. (1989)
Pickett, B.W, et al., 1976. Factors influencing the fertility of stallion spermatozoa in an A.I. program. Proc. 8th Internat. Congr. Anim. Reprod. A.I. Krakow, Poland. 4: 1049 - 1052.
Pickett, B.W. and Back, D.G. 1973. Procedures for preparation, collection, evaluation and insemination of stallion semen. C.S.U. Exp. Sta. Artira. Reprod. Lab. Gen. Series Bull. 935.
Pickett, B.W., and Shiner, K.A., "Recent developments in artificial insemination in horses", Livestock Production Science, 40, 1994, pp 31 - 36.
Pickett, B.W., Burwash, L.D., Voss, J.L. and Back, D.G. 1975b. Effect of seminal extenders on equine fertility. J. Anim. Sci. 40:1136-1143.
Pinkel, D., et al, "Flow Cytometric Determination of the Proportions of X- and Y- Chromosome-Bearing Sperm in Samples of Purportedly Separated Bull Sperm", Journal of Animal Science, Vol. 60, No. 5, 1985, pp 1303 - 1307.
Pinkel, D., Gledhill, B.L., Van Dilla, M.A., Stephenson, D. and Watchmaker, G. 1982b. High resolution DNA measurements of mammalian spermatozoa. Cytometry. 3:1-9. (1982b)
Polge, E. J., "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen, IVF Procedures", Proceedings of the 16th Technical Conference on Artificial Insemination & Reproduction, Cambridge, England, 1996, pp. 7-11.
Purvis, H. T. and J. C. Whittier. "Effects ofionophore feeding and anthelmintic administration on age and weight at puberty in spring-bom beef heifers." J. Anim. Sci. 74:736-744. 1996
Randel, R. D. "Nutrition and postpartum rebreeding in cattle." J. Anim. Sci. 68:853. 1990
Rath, D., et al., "Low Dose Insemination Technique in the Pig", Boar Semen Preservation IV, 2000, pp. 115-118.
Rath, D., et al., "Production of Piglets Preselected for Sex Following in Vitro Fertilization with X and Y Chromosome-Bearing Spermatozoa Sorted by Flow Cytometry", Theriogenology, 47, 1997, pp 795 - 800.
Reiling, B.A., et al., "Effect of Prenatal Androgenization on Performance, Location, and Carcass and Sensory Traits on Heifers in Single Calf Heifer System", J. Anim. Sci., 1995, 73: 986, pp 986-992.
Rens, W., et al, "A Novel Nozzle for More Efficient Sperm Orientation to Improve Sorting Efficiency of X and Y Chromosome-Bearing Sperm", Cytometry 33, 1998, pp. 476-481
Rens, W., et al., "Improved Flow Cytometric Sorting of X- and Y- Chromosome Bearing Sperm: Substantial Increase in Yield of Sexed Semen", Molecular Reproduction and Development, 1999, pp 50-56.
Rieger, D., et al, "The Relationship Between the Time of First Cleavage of Fertilized Cattle Oocytes and Their Development to the Blastocyst Stage", Theriogenology, 1999, pp. 190.
Ritar, A. and Ball, A. 1991. Fertility of young cashmere goats after laparoscopic insemination. J. Agr. Sci. 117:271-273.
Roberts, J.R. 1971. In: *Veterinary Obstetrics and Genital Diseases.* Ithaca, New York. pp. 740-749.
Romita, A. "Some considerations on the beef situation in Italy." (Ed.) J.C. Taylor. The early calving of heifers and it's impact on beef production. 23. 1975
Roth, T.L., Wolfe, B.A., Long, J.A., Howard, J. and Wildt, D.E. 1997. Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. Bio Reprod. 57:165-171.
Roux, M., J. H. Teissier, J. Bonnemaire, and R. Dumont. "Early calving heifers versus maiden heifers for beef -production from dairy herds. 1." The effects of genotype (Friesian and Charolais x Friesian) and 2 feeding levels in the rearing period on growth and carcass quality. Livestock Prod. Sci.16:1. 1987
Rowley, H-S., Squires, E.L. and Pickett, B.W. 1990. Effect ofinsemination volume on embryo recover}' in mares. J. Equine Vet. Sci. 10:298-300.
Roy, J. H. B. "Rearing dairy-herd replacements." J. of the Soc. ofDairy Technology 31:73-79. 1978
Rutter, L. M., et al., "Effect of abomasal infusion of propionate on the GnRH-induced luteinizing-hormone release in prepuberal heifers." J. Anim. Sci. 56:1167. 1983
Salamon, S. 1976. *Artificial Insemination of Sheep.* Chippendale, New South Whales. Publicity Press. p.83-84.
Salisbury, G.W. and VanDemark, N.L. 1961. *Physiology of Reproduction and Artificial Insemination of Cattle.* San Francisco: Freeman and Company.

SAS, SAS/STAT, "Useres Guide (Release 6.03)", SAS Inst. Inc., Cary, NC., 1988. 3 pages
SAS. "The SAS System for Windows." Ver 7.0. Rel 6.12. SAS Inst.Inc., Cary, NC. 2000
Schenk, J. L., T. K. Suh, D. G. Cran, and G. E. Seidel. "Cryopreservation of flow-sorted bovine spennatozoa." Therio. 52:1375. 1999
Schenk, J.L. and Seidel, Jr., G.E., "Imminent Commercialization of Sexed Bovine", Proceedings, The Range Beef Cow Symposium XVL, 1999, pp 89-96.
Schillo, K. K., J. B. Hall, and S. M. Hileman. "Effects of nutrition and season on the onset of puberty in the beef heifer." J. Anim. Sci. 70:3994. 1992
Schmid R.L., et al, "Fertilization with Sexed Equine Spermatozoa Using Intracytoplasmic Sperm Injection and Oviductal Insemination", 7th International Symposium On Equine Reproduction, pp. 139 (Abstract) (1998)
Schnell, T. D., K. E. Belk, J. D. Tatum, R. K. Miller, and G. C. Smith. "Performance, carcass, and palatability traits for cull cows fed high-energy concentrate diets for 0, 14,28,42, or 56 days." J. Anim. Sci. 75:1195. 1997
Schoonmaker, J. P., et al., "Effects of age at weaning and implant strategy on growth of steer calves." J. Anim. Sci. (Suppl2) 76:71 (Abstr.). 1998
Seidel, G. E. and L. A. Johnson. "Sexing mammalian spenn -overview." Therio. 52: 1267. 1999
Seidel, G. E., "Insemination of heifers with sexed sperm." Therio. 52:1407. 1999
Seidel, G.E. Jr., "Uterine Horn Insemination of Heifers With Very Low Numbers of Nonfrozen and Sexed Spermatozoa", Atlantic Breeders Cooperative, Theriogenology 48: pp. 1255-1264, (1997)
Seidel, G.E. Jr., Cran, D.G., Herickoff, L.A., Schenk, J.L., Doyle, S.P. and Green, R.D. 1999. Insemination of heifers with sexed frozen or sexed liquid semen. Theriogenology. 51. (in press). abstr.(1999)
Seidel, G.E., Jr., et al, "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Diary, Loveland, CO, USA January 1996.
Seidel, G.E., Jr., et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa." , Colorado State University, Fort Collins, Atlantic Breeders Cooperative, Lancaster, PA, DUO Dairy, Loveland, CO, July 1996.
Seidel, Jr., G. E., et al, "Insemination of Holstein Heifers With Very Low Numbers Of Unfrozen Spermatozoa", Colorado State University, Atlantic Breeders Cooperative, (1995)
Seidel, Jr., G.E.et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa", Colorado State University (1996)
Sell, R. S., D. L. Watt, R. D. Little, and T. A. Petry. "Single-calfheifer profitability compared to other north dakota beef production systems." Department of Ag. Eco., North Dakota State University, Ag. Econ. Rpt. 20.
Senger, P.L., Becker, W.C., Davidge, S.T., Hillers, J.K. and Reeves, J.J. 1988. Influence of comual insemination on conception rates in dairy cattle. J Anim. Sci. 66:3010-3016.
Shackelford, S. D., M. Koohmaraie, and T. L. Wheeler. "Effects of slaughter age on meat tenderness and usda carcass maturity scores of beef females." I. Anim. Sci. 73:3304. 1995
Shelton, J.N. and Moore, N.W. 1967. The response of the ewe tot pregnant mare gonadotropin and to horse anterior pituitary extract. J. Reprod. Fert. 14:175 - 177.
Shilova, A.V., Platov, E.M. and Lebedev, S.G. 1976. The use of human chorionic gonadothrophin for ovulation date regulation in mares. VIIIth Int. Congr. On Anim. Repro. and A.I. 204-208.
Shorthose, W. R. and P. V. Harris. "Effect of animal age on the tenderness of selected beef muscles." I. Food Sci. 55:1-. 1990
Silbennann, M., "Honnones and Cartilage. Cartilage: development, differentiation, and growth." pp. 327-368. Academic Press, Inc. 1983
Simon, M., "The effect of management option on the perfonnance of pregnant feedlot heifers." M.S. Thesis. Kansas State University. 1983
Smith, G. C., B. W. Berry, J. W. Savell, and H. R. Cross. "USDA maturity indexes and palatability ofbeefrib steaks." J. of Food Quality 11 :1. 1988
Smith, G. C., et al., "Relationship ofusda maturity groups to palatability of cooked beef." J. of Food Sci. 47:1100. 1982
Squires, E., "Simultaneous Analysis of Multiple Sperm Attributes by Flow Cytometry□, Diagnostic Techniques and Assisted Reproductive Technology, The Veterinary Clinics of North America, Equine Practice, Vol. 12, No. 1, April 1996, pp127 - 130.

Squires, E.L, Moran, D.M., Farlin, ME., Jasko, D.J., Keefe, T.J., Meyers, S.A., Figueiredo, E., McCue, P.M. and Jochle, W. 1994. Effect of dose of GnRH analogue on ovulation in mares. Theriogenology. 41:757-769.
Squires, E.L., "Early Embryonic Loss in Equine Diagnostic Ultrasonography", 1st Ed. pp 157-163 Eds Rantanen & McKinnon. Williams and Wilkins, Baltimore, Maryland (1998)
Squires, E.L.., et al, "Cooled and frozen stallion semen", Bulletin No. 9, Colorado State University, Ft. Collins, CO. (1999)
Stellflug, J. N., D. K. Ran, R. D. Randel, and Eo L. Moody. "Plasma estrogens in peri-parturient cow." Therio 10:269. 1978
Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. Lefever. "Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beefheifers." J. Anim. Sci. 74:729. 1996
Story, C. E., R. J. Rasby, R. T. Clark, and C. T. Milton. "Age of calf at weaning of spring-calving beef cows and the effect on cow and calf perfomlance and production economics." J. Anim. Sci. 78:1403. 2000
Sullivan, J.J., Parker, W.G. and Larson, LL. 1973. Duration of estrus and ovulation time in nonlactating mares given human chorionic gonadotropin during three successive estrous periods. J.A.V.M.A. 162:895-898.
Swanson, E. W. "Future research on problems of increasing meat production by early calving." Comm. Eur. Commun., Eur. 5545.1975. The Early Calving ofHeifers and its Impact on Beef Production.
Taljaard, T.L., Terblanche, S.J., Bertschinger, H.J. and Van Vuuren, L.J. 1991. The effect of the laparoscopic insemination technique on the oestrus cycle of the ewe. J. S Afr. Vet. Assoc. 62(2):60-61.
Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. "Carcass characteristics, time on feed and cooked beef palatability attributes." J. Anim. Sci. 50:833. 1980
Taylor, C.S., Moore, A.J. Thiessen, R.B. and Bailey, C.M., AFRC Animal Breeding Research Organisation, West Mains Road, Edinburg EH9 3JQ, "Efficiency of Food Utilization in Traditional and Sex-Controlled Systems of Beef Production", pp 401-440.
Taylor, S. C. S., A. J. Moore, R. B. Thiessen, and C. M. Bailey. "Efficiency of food utilization in traditional and sex-controlled systems of beef-production." Animal Production 40:401. 1985
Tervit, H.R., et al., "Successful Culture In Vitro of Sheep and Cattle Ova", Agricultural Research Council, Unit of Reproduction Physiology and Biochemistry, University of Cambridge, 1972, p. 493-497.
Unruh, J. A. "Effects of endogenous and exogenous growth-promoting compounds on carcass composition, meat quality and meat nutritional-valu~." J. Anim. Sci. 62:1441. 1986
US Application , 09/454,488, entitled "Improved Flow Cytometer Nozzle and Flow Cytometer Sample Handling Methods", filed December 3, 1999.
US Application , 60/238,294, entitled "Hysteroscopic Insemination of Mares" filed October 5, 2000.
US Application , 09/448,643, entiled "Multiple Sexed Embryo Production System for Mammals", filed November 24, 1999.
US Application , 09/511,959 entitled "Methods For Improving Sheath Fluids and Collection Systems For Sex-Specific Cytometer Sorting of Sperm", filed February 23, 2001.
US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.
US Application 09/015, 454, entitled "System for Improving Yield of Sexed Embryos in Mammals", filed on January 29, 1998, 59 total pages which includes drawings.
US Application 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.
US Application entitled "System For Separating Frozen-Thawed Sperm Cells Into X-Chromosome And Y-Chromosome Bearing Populations", filed November 28, 2000.
US Application Serial Number 60/094,720, entitled "System for Low Dose Insemination of Equines", filed July 30, 1998.
US Application Serial Number 60/113,143, entitled "Equine Insemination System", December 18, 1998.
US Application Serial Number 60/203,089, entitled "Detector System for Resolving Small Differences in Photo-generated Signal", filed May 9, 2000.
US Application Serial Number 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.
US Application Serial Number 60/224,050., entitled "Integrated System for Herd Management With Terminal-Cross Program Using Sexed Semen", filed August 9, 2000.
USDA "Official United States standards for grades of carcass beef." Agric, Marketing Serv., USDA .Washington,

DC. 1997
Vazquez, J. et al., "Nonsurgical Uterotubal Insemination in the Mare", Proceedings of the 44th Annual Convention of the American Association of Equine Practitioners, Baltimore, Maryland, December 6-9, 1998, Vol. 44, pp 68-69
Vazquez, J., et al., "A.I. in Swine; New Strategy for Deep Insemination with Low Number of Spermatozoa Using a Non-surgical Methodology", 14th International Congress on Animal Reproduction, Vol. 2, Stockhlom, July, 2000, p. 289.
Vazquez, J., et al., "Development of a Non-surgical Deep Intra Uterine Insemination Technique", IV International Conference on Boar Semen Preservation, Maryland, August, 1999, p 35 and photo of display board.
Vazquez, J., et al., "Successful Low-Dose Insemination by a Fiberoptic Endoscope Technique in the Sow ", Proceedings Annual Conference of the International Embryo Transfer Society, Netherlands, Theriogenology, Vol. 53, January, 2000, pp. 201.
Vazquez, J., et al.,"Hypoosmotic Swelling Test as Predictor of the Membrane Integrity in Boar Spermatozo", Boar Semen Preservation IV, IVth International Conference on Boar Semen Preservation, Maryland, pp. 263.
Vidament, M., Dupere, A.M., Julienne, P., Evain, A., Noue, P. and Palmer, E. 1997. Equine frozen semen freezeability and fertility field results. Theriogenology. 48:907.
Vincent, B. C., S. D. M. Jones, L. E. Jeremiah, M. A. Price, and J. A. Newman. "Carcass characteristics and meat quality of once-calved heifers." Canadian J. Anim. Sci. 71:311. 1991
Voss, J.L. and Pickett, B.W. 1976. Reproductive management of the broodmare. C.S.U. Exp. Sta. Anim. Reprod. Lab. Gen. Series. Bull. 1-12
Voss, J.L., Pickett, B.W., Burwash, L.D. and Daniels, W.H. 1974. Effect of human chorionic gonadotropin on duration of estrous cycle and fertility of normally cycling, nonlactating mares. J.A.V.M.A. 165:704-706.
Voss, J.L., Squires, E.L., Pickett, B.W., Shideler, R.K. and Eikenberry, D.J. 1982. Effect of number and frequency of inseminations on fertility in mares. J. Reprod. Fertil. Suppl. 32:53-57.
Waggoner, A. W., M. E. Dikeman, I. R. Brethour, and K. E. Kemp. "Performance, carcass, cartilage calcium, sensory and collagen traits of longissimus muscles of open versus 30-month-old heifers that produced one calf." I. Anim. Sci. 68:2380. 1990
Welch G.R., et al., 1994. Fluidic and optical modifications to a FACS IV for flow sorting of X- and Y-chromosome bearing sperm based on DNA. Cytometry 17 (suppl. 7): 74.
Welch, G., et al., "Flow Cytometric Sperm Sorting and PCR to Confirm Separation of X- and Y- Chromosome Bearing Bovine Sperm□, Animal Biotechnology, 6 (2), 131-139, 1995, pp 131 - 139.
Wheeler, T. L., L. v. Cundiff, and R. M. Koch. "Effect of marbling degree on beef palatability in Bos- Taurus and Bos-Indicus cattle." J. Anim. Sci. 72:3145. 1994
Wickersham, E. W. and L. H. Schultz. "Infilience of age at first breeding on growth, reproduction, and production ofwell-fed holstein heifers." J. Dairy Sci. 46:544. 1963
Wilson, C.G., Downie, C.R., Hughes, J.P. and Roser, J.F. 1990. Effects of repeated hCG injections on reproductive efficiency in mares. Eq. Vet. Sci. 4:301-308.
Wilson, M.S. 1993. Non-surgical intrauterine artificial insemination in bitches using frozen semen. J.Reprod. Fert Suppl. 47:307-311.
Woods, J. and Ginther, O.J. 1983. Recent studies related to the collection of multiple embryos in mares. Theriogenology. 19:101 - 108.
Woods, J., Bergfelt, D.R. and Ginther, O.J. 1990. Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares. Eq. Vet. J. 22(6):410-415.
XP-002103478, File Biosis, one page.

In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary,

second edition are hereby incorporated by reference. However, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

In addition, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like.

Thus, the applicant(s) should be understood to have support to claim at least: i) each of the staining, separation, isolation, insemination, or fertilization procedures as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, and x) the various combinations and permutations of each of the elements disclosed.

The claims set forth in this specification are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the

description into the claims or vice-versa as necessary to define the subject matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

VI. CLAIMS

I claim:

1. A method of transporting oocytes, comprising the steps of :
   a. collecting oocytes from a female mammal;
   b. entraining oocytes in a fertilization medium;
   c. transferring oocytes in said fertilization medium into a straw;
   d. sealing said straw containing said oocytes;
   e. transferring at least one straw inside of an incubation element;
   f. establishing incubation conditions within said incubation element;
   g. sealing said incubation element;
   h. transporting said oocytes from said female mammal within said straw inside of said incubation element.

2. A method of transporting oocytes as described in claim 1, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.

3. A method of transporting oocytes as described in claim 1, wherein said fertilization medium comprises modified Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20μg heparin per milliliter of Tyrode's medium, and a concentration of 5 milli-molar caffeine.

4. A method of transporting oocytes as described in claim 1, wherein between about 10 and about 15 of said oocytes are contained within about 50 micro-liters of said fertilization medium.

5. A method of transporting oocytes as described in claim 1, wherein said straw has heat sealable aperture elements.

6. A method of transporting oocytes as described in claim 5, wherein said straw has a interior volume of about 0.25 milliliters.

7. A method of transporting oocytes as described in claim 1, wherein said incubation element has sealable aperture elements.

8. A method of transporting oocytes as described in claim 7, wherein said incubation element comprises a glass tube.

9. A method of transporting oocytes as described in claim 1, wherein said incubation conditions comprise an atmosphere of 5 percent carbon dioxide in air and a temperature of 39 degrees Centigrade within said incubation element.

10. A method of transporting oocytes as described in claim 1, further comprising the step of transferring sperm cells to said straw containing said oocytes in said fertilization medium.

11. A method of transporting oocytes as described in claim 10, wherein said step of transferring sperm cells to said straw containing said oocytes comprises establishing a concentration of sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.

12. A method of transporting oocytes as described in claim 1, further comprising the step of separating spermatozoa into enriched X-chromosome bearing and Y-chromosome populations.

13. A method of transporting oocytes as described in claim 12, further comprising the step of transferring separated sperm cells to said straw containing oocytes.

14. A method of transporting oocytes as described in claim 13, wherein said step of transferring separated sperm cells to said straw containing oocytes comprises establishing a concentration of said separated sperm cells in said fertilization

medium of about 1 million to about 2 million per milliliter of fertilization medium.

15. A method of transporting oocytes as described in claims 10, 11, 12, 13, or 14 further comprising the step of transferring said straw containing said oocytes in said concentration of said sperm cells to said incubation element.

16. A method of transporting oocytes as described in claim 15, further comprising the step of establishing fertilization conditions within said incubation element.

17. A method of transporting oocytes as described in claim 16, wherein said step of establishing fertilization conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.

18. A method of transporting oocytes as described in claim 16, further comprising the step of transporting said oocytes in said fertilization conditions.

19. A method of transporting oocytes as described in claim 18, further comprising the step of fertilizing at least some of said oocytes during transport.

20. A method of fertilizing oocytes, comprising the steps of:
    a. collecting sperm cells from a male mammal;
    b. transferring said sperm cells to a straw containing a plurality of oocytes in fertilization medium, wherein said oocytes are from a female mammal of the same species as said male mammal;
    c. sealing said straw containing said sperm cells and said oocytes;
    d. transferring said straw containing said sperm cells and said oocytes to the inside of an incubation element;
    e. establishing incubation conditions within said incubation element;
    f. sealing said incubation element; and

g. fertilizing at least one oocyte in said straw.

21. A method of transporting oocytes as described in claim 20, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.

22. A method of transporting oocytes as described in claim 20, wherein said fertilization medium comprises modified Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20μg heparin per milliliter of Tyrode's medium, and a concentration of 5 milli-molar caffeine.

23. A method of transporting oocytes as described in claim 20, wherein between about 10 and about 15 of said oocytes are contained within about 50 micro-liters of said fertilization medium.

24. A method of transporting oocytes as described in claim 20, wherein said straw has heat sealable aperture elements.

25. A method of transporting oocytes as described in claim 24, wherein said straw has a interior volume of about 0.25 milliliters.

26. A method of transporting oocytes as described in claim 20, wherein said incubation element has sealable aperture elements.

27. A method of transporting oocytes as described in claim 26, wherein said incubation element comprises a glass tube.

28. A method of transporting oocytes as described in claim 20, wherein said incubation conditions comprise an atmosphere of 5 percent carbon dioxide in air and a temperature of 39 degrees Centigrade within said incubation element.

29. A method of transporting oocytes as described in claim 20, further comprising the step of transferring said sperm cells to said straw containing said oocytes in said fertilization medium.

30. A method of transporting oocytes as described in claim 29, wherein said step of transferring sperm cells to said straw containing said oocytes comprises establishing a concentration of sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.

31. A method of transporting oocytes as described in claim 21, further comprising the step of separating said sperm cells into enriched X-chromosome bearing and Y-chromosome populations.

32. A method of transporting oocytes as described in claim 31, further comprising the step of transferring separated sperm cells to said straw containing oocytes.

33. A method of transporting oocytes as described in claim 32, wherein said step of transferring separated sperm cells to said straw containing oocytes comprises establishing a concentration of said separated sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.

34. A method of transporting oocytes as described in claims 29, 30, 31, 32, or 33 further comprising the step of transferring said straw containing said oocytes in said concentration of said sperm cells to said incubation element.

35. A method of transporting oocytes as described in claim 34, further comprising the step of establishing fertilization conditions within said incubation element.

36. A method of transporting oocytes as described in claim 35, wherein said step of establishing fertilization conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37

degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.

37. A method of transporting oocytes as described in claim 35, further comprising the step of transporting said oocytes in said fertilization conditions.

38. A method of transporting oocytes as described in claim 37, further comprising the step of fertilizing at least some of said oocytes during transport.

39. A method of transporting oocytes as described in claim 37, further comprising the step of removing fertilized oocytes from said straw.

40. A method of transporting oocytes as described in claim 37, further comprising the step of implanting said fertilized oocytes into a female mammal.

41. A method of transporting oocytes as described in claim 37, wherein said step of implanting said fertilized oocytes into a female mammal comprises implanting said fertilized oocytes into the same species of said female mammal as said male mammal.

42. A method of transporting oocytes as described in claim 37, wherein said step of implanting said fertilized oocytes into female mammal comprises implanting fertilized oocytes into a different species of said female mammal then said male mammal.

43. A mammal having predetermined sex produced according to claims 35, 37, 38, 39, 40, 41, or 42.

44. A portable incubation system, comprising:

a. a straw configured to contain fertilization medium into which a plurality of oocytes from a female mammal and sperm cells from a male mammal are transferred;

b. an incubator element configured to encapsulate said straw containing fertilization medium into said oocytes from said female and said sperm cells from said male mammal have been transferred; and

c. an incubation environment maintained within said incubator element, comprising:

i. an incubation atmosphere of about 5 per cent carbon dioxide in air; and

ii. a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade.

45. A portable incubation system as described in claim 44, wherein said mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.

46. A portable incubation system as described in claim 44, wherein said fertilization medium comprises Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20 μg heparin per milliliter of fertilization medium, and a concentration of 5 millimolar caffeine.

47. A portable incubation system as described in claim 44, wherein said plurality of oocytes from a female mammal comprises about 10 oocytes to about 15 oocytes per 50 microliters of fertilization medium.

48. A portable incubation system as described in claim 44, wherein said sperm cells from said male mammal have a concentration of about 1 million to about 2million per milliliter of said fertilization medium.

49. A portable incubation system as described in claim 44, wherein said sperm cells comprise sperm cells separated on the basis of bearing an X-chromosome or bearing a Y-chromosome.

50. A portable incubation system as described in claim 44, wherein said incubation element comprises glass tube.

51. A portable incubation system as described in claim 44, wherein said incubation environment is maintained between about 18 hours and about 20 hours.

52. A portable incubation system as described in claim 44, further comprising a transportation element to move said portable incubation system the distance between two locations.

53. A portable incubation system as described in claim 52, wherein fertilization of said oocytes from a female with said sperm cells from said male mammal within said fertilization medium contained by said straw occurs during transport of said portable incubator system.

54. A portable incubation system as described in claim 53, wherein said two locations comprise a sperm cell separation facility having a first location and a female mammal into which fertilized oocytes are implanted having a second location.

55. A portable incubation system as described in claim 44, wherein said a straw configured to contain fertilization medium into which a plurality of oocytes from a female mammal and sperm cells from a male mammal are transferred has a volume of 0.25 milliliters.

56. A method of in vitro fertilization of oocytes, comprising the steps of :

a. collecting oocytes from a female mammal;

b. entraining a plurality of oocytes in a fertilization medium containing both essential and non-essential amino acids:

c. inseminating said oocytes with sperm cells collected from a male mammal of the same species as said female mammal;

d. establishing inseminated oocytes in incubation conditions within an incubation element; and

e. fertilizing at least one of said oocytes.

57. A method of in vitro fertilization of oocytes as described in claim 56, wherein said fertilization medium is selected from the group consisting of chemically defined medium, SOF, or TALP supplemented with Eagles Medium.

58. A method of in vitro fertilization of oocytes as described in claim 56, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.

59. A method of in vitro fertilization of oocytes as described in claim 56, wherein said non-essential amino acids have a concentration in said fertilization medium are futher supplemented with essential amino acids.

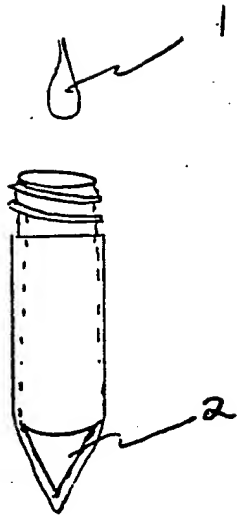
60. A method of in vitro fertilization of oocytes as described in claim 56, entraining a plurality of oocytes in a fertilization medium containing both essential and non-essential amino acids comprises entraining about 10 oocytes to about 15 oocytes in about 50 microliters of fertilization medium.

61. A method of in vitro fertilization of oocytes as described in claim 56, wherein said step of establishing oocytes in incubation conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.

62. A method of in vitro fertilization of oocytes as described in claim 56, wherein said step of inseminating said oocytes with sperm cells collected from a male mammal of the same species as said female mammal comprises establishing a concentration of said separated sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.

63. A method of in vitro fertilization of oocytes as described in claim 56, wherein said sperm cells are separated on the basis of bearing an X-chromosome or bearing a Y-chromosome.

FIG 1



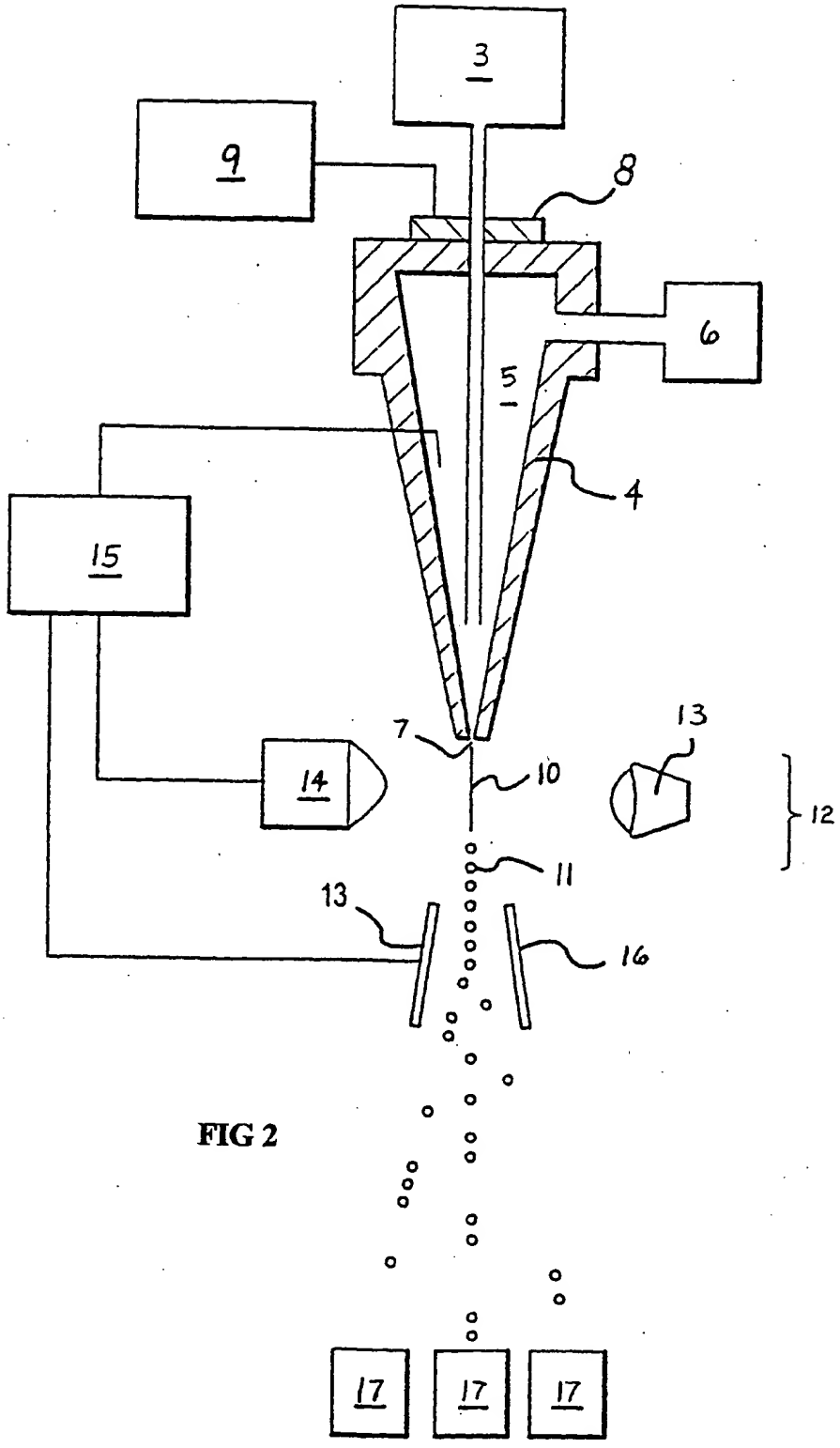


FIG 2

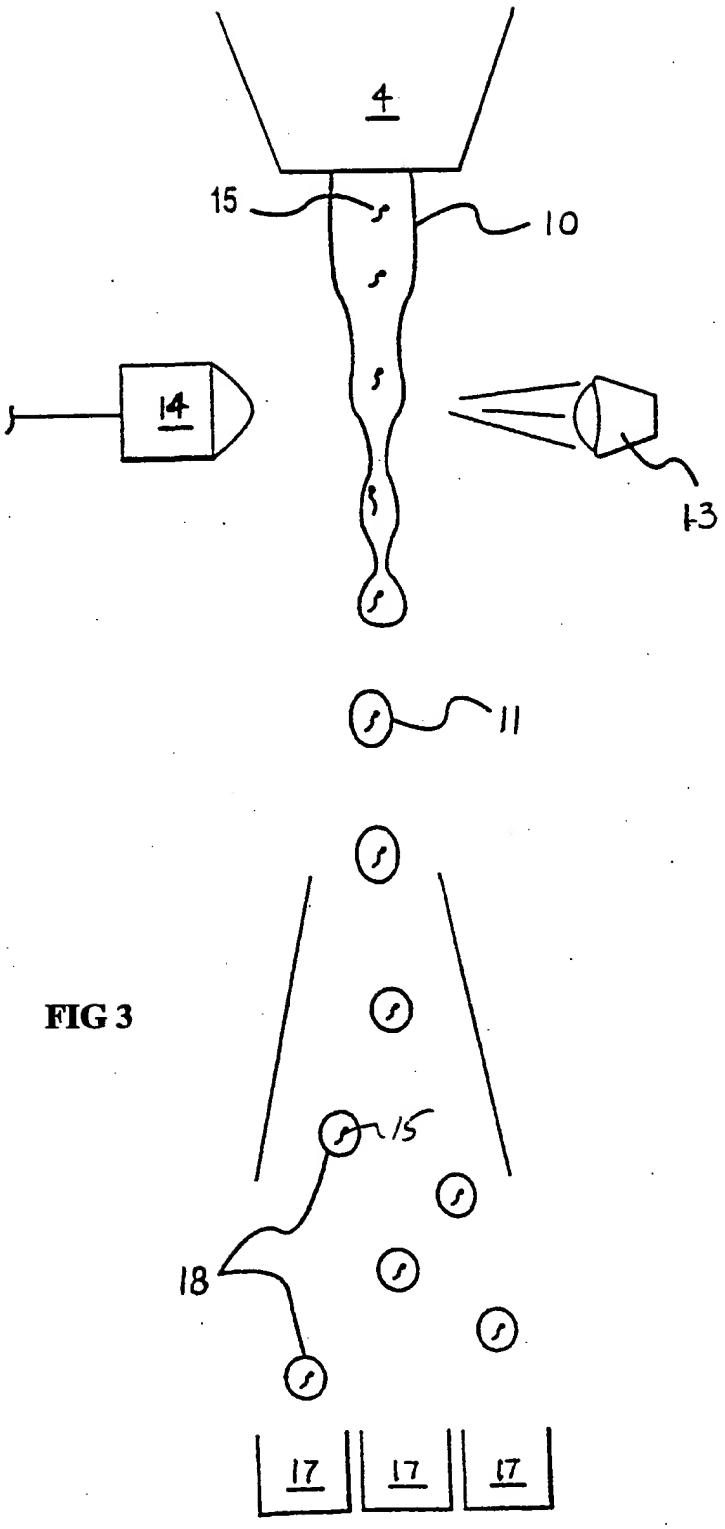


FIG 3

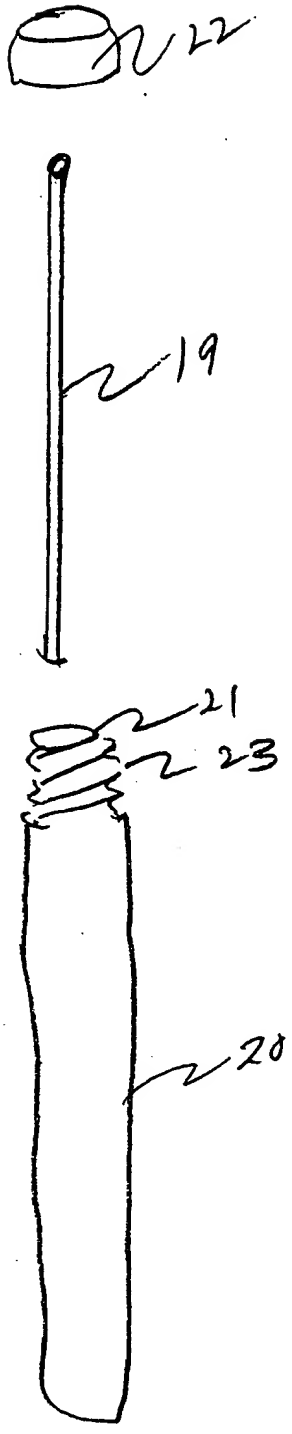


FIG 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/45237

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 1/00
US CL : 435/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPATFULL, MEDLINE, BIOSIS, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,119,465 A (MULLENS et al.) 19 september 2000.	1-19
A,P	US 6,238,920 B1 (NAGAI et al.) 29 May 2001.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 April 2002 (19.04.2002)

Date of mailing of the international search report
14 MAY 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703)305-3230

Authorized officer
Sandra Saucier
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/45237

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-19

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ *No protest* accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-19, drawn to a first method, a method of transporting oocytes.

Group II, claims 20-42, drawn to a second method, a method of fertilizing oocytes.

Group III, claim 43, drawn to a mammal of a preselected gender.

Group IV, claims 44-55, drawn to an incubation system.

Group V, claims 56-63 drawn to a third method, a method of in vitro fertilizing of oocytes. The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, II and V are directed to three different methods because they have distinct steps. Multiple methods are not permitted under PCT Rule 13.1.

Group III does not relate to a single general inventive concept under PCT Rule 13.2 because sex selection of mammals is well known in the prior art and further, the animal, per se, cannot be distinguished from an animal whose gender was not predetermined.

Group IV is not related to a single general inventive concept under PCT Rule 13.2 because the incubation system of Group IV where the temperature and CO_2 levels are stipulated is not required to be used in the methods of Groups I, II or V where the methods of claims 1, 20 or 56 do not stipulate any temperature or gas concentration.